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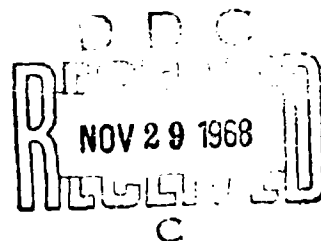
STUDIES ON THE TUBERCULAR REACTION AND ITS SECONDARY
EFFECTS ON THE RHESUS MONKEY (MACACA MULATTA)

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schaften), Berlin (Director: [The
late] Prof. Dr. J. Dobberstein).

With 9 figures

(Manuscript received 16 March 1966)

The most frequently employed method in sub-human primates for tu-
berculosis study is the skin-sensitivity test with tuberculin. It has been
established in earlier studies on tuberculosis and the tuberculin reaction
in monkeys (RUCH 1959; TROLLDENIER 1964, 1965) that the investigators in
past times used widely different tuberculin concentrations and that these
concentrations were usually further increased if the results were negative,
i. e., if no positive reaction was evident in tuberculotic animals. As a
result, there were considerable differences with respect to the concentra-
tion of the injection dose in the investigations by various reporters.

We have therefore conducted a study with increasing and decreasing
concentrations of the purified tuberculin (P.P.D.), eliciting tuberculin
reactions in rhesus monkeys that were exposed to natural tuberculosis in-
fection and to determine the optimum tuberculin concentration. In these
studies, tests were made to establish whether the skin over different areas
of the body would exhibit different degrees of sensitivity. At the same
time, we established the titer of humoral antibodies by means of the hem-
agglutination reaction (HAR) of MIDDLEBROOK and DUBOS prior to and following

the tuberculin test within brief specific periods, and the dependence of this on the tuberculin concentration and the interval after the injection. To establish additional humoral consequences of the tuberculin test, we determined the blood-cell values and, in some instances, the serum protein fractions.

Materials and Methods

Six adult Rhesus monkeys (five females and one male) were available for the study. Four of these animals (Nos. 1-4) represented the remainder of a Rhesus monkey group that was annihilated by tuberculosis (TROLLDENIER 1964); these animals exhibited in many instances a positive tuberculosis reaction before the start of the investigation. They received five months prior daily doses of 50 mg INH in the fodder for a period of 30 days. The section of these four spontaneously tuberculosis-infected monkeys, three of which died in agony, showed in animals No. 2, 3, and 4, general tuberculosis with caseous scatter herds mainly in the spleen, liver, lung, and serosa. Acid-fast rods were identified in all modified organs. Sensitivity tests of the isolated tuberculosis strains conducted at the Research Institute for Tuberculosis and Pulmonary Disease (Institut fuer Tuberkulose und Lungenkrankheiten), Berlin-Buch (Director: Professor Dr. STEINBRUECK), showed that almost the entire population of the germs had the normal sensitivity to INH, streptomycin, PAS, ethioniamide, cycloserine, and thiosemicarbazone. The Typus humanus was identified on the basis of the positive niacine test.

Monkey No. 1, which survived all other tuberculotic animals, showed after being killed a pin-head sized yellow herd at the dorsal lung surface and a small number of similar changes at the surface of the liver. In stained smear and section preparates, no acid-fast rodlets could be identified; however, there was a strong connective tissue demarcation with leucocytal infiltration, epitheloidal cell clusters, and LANGERHANS-type giant cells and also Cossa-positive central calcified deposits.

Rhesus monkey No. 5, exhibiting a generally decreasing lymphatic leucocytosis, showed no clinical changes and was considered as healthy. For a period of 14 months after the end of the test, it exhibited no symptoms of disease, especially no evidence of progressing tuberculosis.

In Rhesus monkey No. 6, there developed within a few months an ascites with 140 ml of yellow transsudate. General tuberculosis was proven in the autopsy, caused by Mycobacterium bovis. Sensitivity tests of the tuberculosis strain isolated from the lung showed a multiple sensitivity decrease against the tuberculostatic agents mentioned above. No therapy has been undertaken.

The tuberculin injections were administered simultaneously in doses of 1, 10, 100, and 1000 at the upper eyelid and the underarm ('Purified

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Tuberculin,' Dessau); with a dose of 5000 TE being administered only in
one of the two injection types. Each animal has been infected in the course
of one year at various periods with various concentrations of tuberculin.
Evaluation of the reaction was undertaken in daily intervals of up to 72
hours and longer p. inj. The reaction was considered as positive in cases
of reddening and edema (Ptosis) in the lid at the same time, occasionally
also necroses, 16 hours p. inj., and cases of reddening, swelling, and in-
creased temperature coupled with non-shiftability of the skin at the under-
arm. Increased thickness of the skin on the arm was in some instances mea-
sured with a cutimeter.

Blood sampling was undertaken from the saphena vein daily or in
three-day intervals at noontime prior to feeding. The erythrocytes and leu-
cocytes were counted in the combined Neubauer chamber. The differential
blood image was established according to the method of PAPPENHEIM or WAHBY
(1964), employing stained smears.

In addition, 126 serum specimens were investigated with the aid of
the hemagglutination reaction according to MIDDLEBROOK and DUBOS: this me-
thod proved satisfactory in earlier studies (SCHROEDER 1964) for the iden-
tification of tuberculosis antibodies in monkeys. For this test, the tu-
berculosis antigen 'Dessau,' specially prepared for it, was employed: this
antigen proved to be comparable against other tuberculins according to
SPECK (1957). Hammel erythrocytes were sensitized in HAR by the effect of
a tuberculosis bacterium extract; i.e., antigens from this extract were adsor-
bed at the surface of the erythrocytes. The sensitized erythrocytes are
agglutinated by antibodies directed against tuberculosis bacteria.

Twenty-four sera were investigated with the aid of paper electro-
phoresis; the specimens being taken daily after the 5000 TE injection for
up to 8 days p. inj. The pherograms were prepared with the VEB Carl Zeiss
Jena separating apparatus on Schleicher-Schuell paper 2043b, using a vero-
nal sodium/sodium acetate buffer (pH = 9.0; ion strength μ = 0.06). The
strips were stained according to GRASSMANN and HANNIG with Amido Black
ERI 10 from VED Carl Zeiss Jena.

In order to determine the injection depth at the upper eyelid more
precisely, the tuberculin was replaced in one animal by the same quantity
of hematoxylin and the injection administered intrapalpebrally under anes-
thesia. A small portion of the dye expanded in the small vessels up to
the two corners of the eye before the injection was even completed. Both
lids were processed histologically.

Results

1. Tuberculin test with 1 TE

The intrapalpebral tuberculin test with 1 TE caused no reaction
in one animal (No. 4) that reacted positively months before and also in

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subsequent tests with higher doses.

2. Tuberculin test with 10 TE

Injections of 10 TE each were administered to two Rhesus monkeys
(Nos. 1 and 4) in the upper eyelid and the shaved skin of the underarm.
The palpebral specimens were negative after 72 hours, whereas a flat spot
developed (without reddening) at the arms of both animals, whereby the
skin thickness increased from the normal 0.9-1.0 mm to 1.7 mm, compared
to the skin in the other arm. The slight increase in the leucocyte number
in animal No. 1 during the 3rd day p. inj. was not accompanied by a shift
in the differential blood image. The hemagglutination titer remained unaf-
fected also (Fig. 1).

Fig. 1. The course of the hemagglutination titer (——), the leu-
cocyte number (— — —), the segment particle number (----), and
the lymphocyte number (-.-.-) for a period of 78 days. The dual
arrows represent simultaneous injections in the eyelid and the
arm with the dose indicated. 1) Relative-percent leucocytes;
2) absolute-percent leucocytes; 3) days.

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3. Tuberculin test with 100 TE

Both animals (Nos. 1 and 4) were subsequently subjected to the tuberculin test with 100 TE per dose at the two injection sites simultaneously. The result was again a clear reaction at the underarm with a swelling of 2.0 and 1.5 mm against a normal skin thickness of 1.2 and 1.0 mm, respectively. The higher skin swelling was accompanied by slight reddening; later, skin scabs were evident at this location. The palpebral specimen was negative in both monkeys 72 hours p. inj; only during the 7th day was there dubious evidence in one animal (No. 4) with reddened but not swollen eyelid. The blood test conducted three days after the tuberculin injection showed a reduction of the leucocytes from 10,400 and 9000 to 7600 and 7000, respectively, combined with a relative increase in lymphocytes by 8-10% and a corresponding relative loss of granulocytes. The blood-cell values returned to normal between the 7th and 10th day; and became somewhat higher than normal in subsequent days. The antibody titer was increased in both monkeys on the 3rd day p. inj. from 1:20 and 1:80 by one dilution step to 1:40 and 1:160, respectively, and returned to the intial value between the 7th and 10th day (Fig. 1).

4. Tuberculin test with 1000 TE

The next tuberculin concentration involved 1000 TE per dose since an equivalent quantity of old tuberculin diluted at the ratio of 1:10 is often quoted as the test dose for monkeys in the Anglo-Saxon literature. The result of the tuberculin tests in the upper eyelid and the skin of the underarm can be classified in two types of reaction. In animals Nos. 1 and 4, who received two doses of 100 TE each 24 days earlier, there was no positive reaction at the lid and at the underarm, apart from a slight reddening of the lid in one of the animals. In the third animal, that was not administered tuberculin before (No. 3), there was a dubious evidence of slight swelling in the right lid (Fig. 2) and a strongly positive reaction at the arm. The inflamed skin was of higher temperature, very red, and could not be shifted with respect to the substrate (Fig. 3). The size was 3 x 2 cm. The skin spot was still 1 x 1 cm in size on the 6th day, slightly red, brittle, and showed two skin necroses 2 x 2 mm in size. Brittle crusts were still evident 24 days later (Fig. 4). The HA titer decreased in animal No. 1 by one degree of dilution to 1:20, whereas it remained unchanged in the other two. All animals exhibited extremely high increases to 1:160 or 1:320 on the 14th day of the experiment: the increase was still evident in some instances during the 17th day.

In spite of difference in the skin reaction, all three animals exhibited the same behavior in the white blood particle test. On the 3rd day p. inj., the leucocyte number increased from 10,000 to 14,000/cu mm on the average, and the segment-grained granulocyte vs lymphocyte ratio increased by 16-24% to 39-65% through the increase in segment-grained particles. Correspondingly, the relative lymphocyte value decreases also. Between the 6th and 14th days, the initial values were again re-established,

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whereby fluctuations in all blood-cell values were still evident in hemograms taken during the next three days.
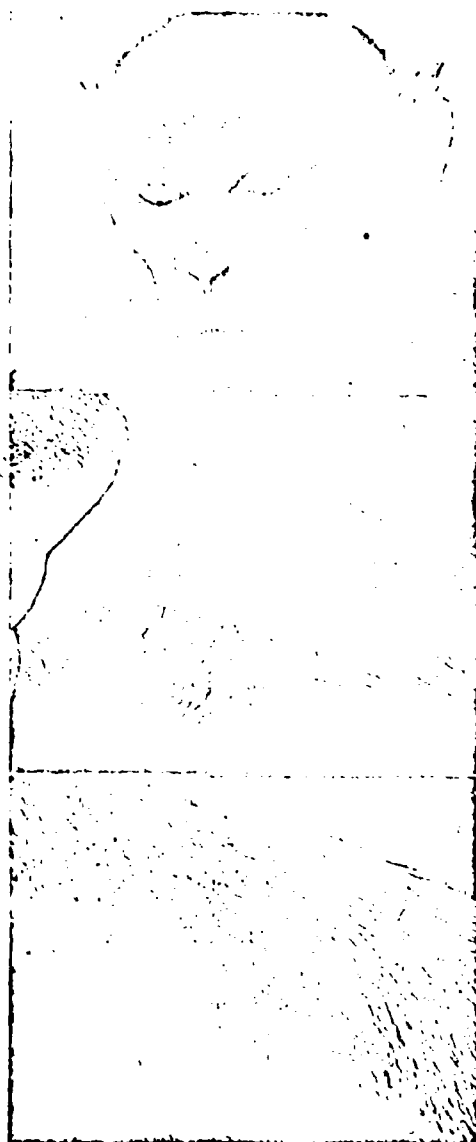
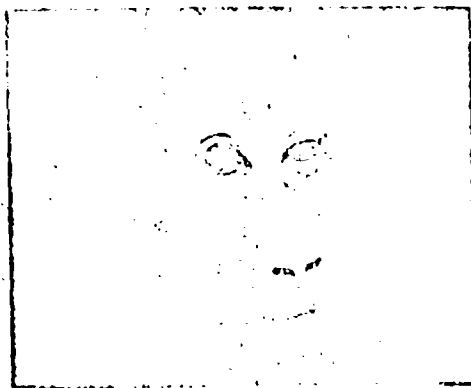Fig. 2. Dubious palpebral reaction with slight swelling in the right lid after 1000 TE, 72 hours p. inj.

Fig. 3. Vigorous, highly reddened tuberculin reaction (extent: 3 x 2 cm) after 1000 TE, 72 hours p. inj., on the underarm

Fig. 4. Intracutaneous reaction with pustules 24 days after 1000 TE

5. Tuberculin test with 5000 TE

All experimental monkeys were subjected to repeated tuberculin tests with 5000 TE per dose. Tuberculinization was undertaken either on the lid or the underarm.

There were differences in the tuberculin reactions. In the eyelids of monkeys Nos. 2, 3, and 6 -- which were not tuberculinized for several weeks or months prior to the test -- there were clearly positive allergy reactions (Fig. 5).

Fig. 5. Clearly positive palpebral reaction with Ptosis in case of 5000 TE, 72 hours after injection.

The reaction was considered as dubious in the case of monkey No. 4 since the swelling and reddening of the lid were slight. This monkey reacted positively two months previously in a test involving subcutaneous administration of 5000 TE in the underarm. Four palpebral tests, initially of a dubious nature, were classed as negative in monkey No. 1. Also, two palpebral tuberculin injections in monkey No. 5 showed no positive results, as was expected on the basis of anamnesis. The course of the titer changes following the palpebral injections with 5000 TE indicated an effect of the tuberculin on the titer level (Fig. 1). In cases of positive palpebral reactions, there was a decrease on the 3rd day p. inj. -- the titer even disappeared altogether in some instances (monkey No. 2) -- whereas it increased beyond the initial value during subsequent days. A delayed reaction was elicited in the moribund monkey No. 6 only 7 days after the tuberculinization. Here, there were no antibodies evident first, and then there were titer values as high as 1:320 (Fig. 6). The titer was 1:160 14 days after the tuberculin test, shortly before death. The serum of this animal showed considerable changes in the serum-protein fraction composition even prior to the tuberculinization. The animal exhibited extreme hypalbuminemia, parallel to a

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hyper-γ-globulinemia, as shown by the following values:

> Albumin: 4.8 relative-percent; Globulin : α 13.5 relative-percent; β, 22.0 relative-percent; γ, 59.7 relative-percent.

This strong loss of albumin, and the defective water-binding ability of the blood connected with it, necessarily resulted in a heavy loading of the circulation (ANTWEILER 1957). In the course of the 8-day test period there was a slight increase in the albumin value to 11.0 relative-percent (on the average) with a simultaneous slight decrease of the γ-globulin value to approximately 5.15 relative-percent; however, the distribution is so extreme here that the basic finding is not affected by this factor (Fig. 7). The increase in titer on the 7th day p. inj. could not be followed on the pherogram.
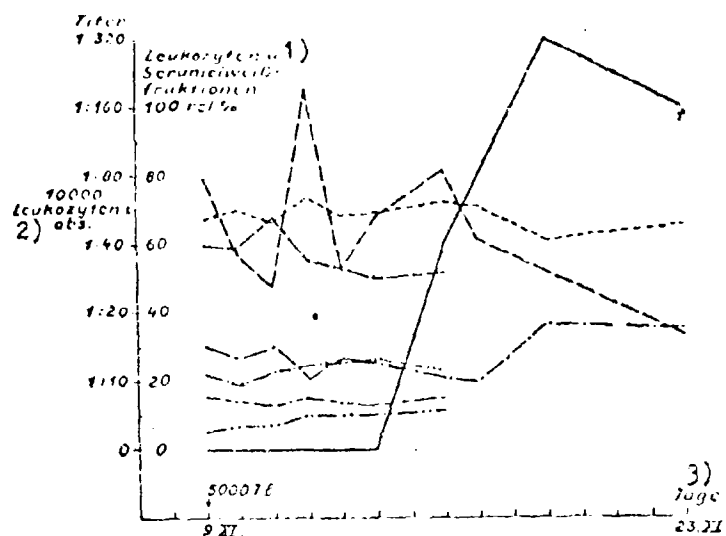
Fig. 6. The course of the hemagglutination titer (——), leucocyte number (— — —), segmented-grain number (- - -), lymphocyte number (-.-.), and serum-protein albumin (— - - —), α-globulin (— - - ·⤳, β-globulin (— - ⋈ - —), and γ-globulin (— ⋈ —) for the 14-day period following injection of 5000 TE. 1) Leucocyte and serum-protein fractions; 2) leucocytes, absolute-percent; 3) days.

In the dubious palpebral reactions of monkeys Nos. 1 and 4, no titer or only a titer of 1:10 was evident on the 3rd day; this increased to 1:160 and 1:40, respectively, during the next seven days.

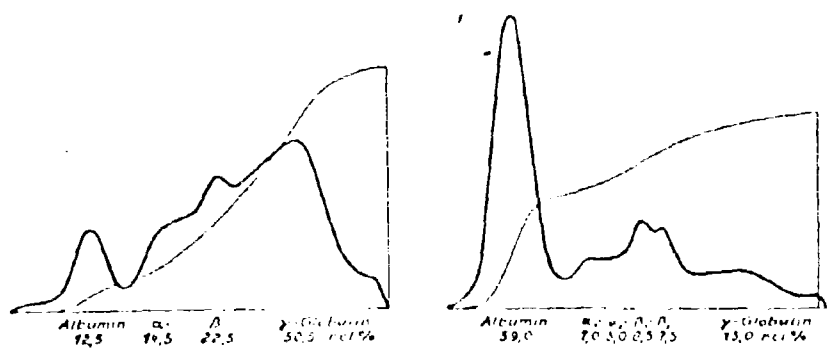Fig. 7 (left). Pherogram of a Rhesus monkey in the breakdown
form of tuberculosis on the 7th day after injection of 5000
TE (see also Fig. 6). The text contains the average values
from several pherograms.
Fig. 8(right). Pherogram of a Rhesus monkey with slightly
spread tuberculosis on the 5th day of the experiment after
injection of 5000 TE. No significant changes from the ini-
tial values.

The negative palpebral injections of monkey No. 1 caused in most
instances no titer changes during the first five days; however, there was
some increase on subsequent days. In spite of the increase in titer, the
serum-protein values obtained by electrophoresis were not significantly
changed in comparison with the initial values during an observation period
of eight days. Any fluctuations, that are typical for monkeys in the nor-
mal case also (SCHERMER 1954, DEUTSCH 1945), were slight and turned out to
be non-specific (Fig. 8).

Changes in the white blood cell image were also observed in the
palpebral tests that were conducted. In most instances, the leucocyte num-
ber decreased by 2-3000/cu mm 72 hours p. inj. The shifts in the differen-
tial blood image exhibited noteworthy correlations with the changes in the
titer values that were established in previous tests with lower tuberculin
concentrations. A percentagewise decrease of the rod- and segment-grained
granulocyte numbers was often accompanied by a decrease of leucocyte num-
ber, whereas a relative increase in lymphocyte number preceded an increase
of titer or ran parallel to it. Accordingly, a decrease of titer was often
accompanied by a relative decrease of the lymphocyte number or the latter
was observed before the reduction of the titer (Fig. 1). These observa-
tions indicate relationships between lymphocyte function and antibody pro-
duction such as described by GRAU (1965).

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Tuberculin reactions on the skin of the underarm were generally more pronounced that those shown in the palpebral reaction, and could also be followed in some instances for a longer period of time. In two animals there was a 3 x 4 cm, or penny-sized, reddish brittle swelling 72 hours after the test: this area could not be shifted with respect to the substrate. As late as the 6th day, the skin thickening was still evident: 2.0-2.4 mm as compared to a normal skin thickness of 1.4 mm. Another animal (No. 1) showed a negative tuberculin test at the underarm and no significant changes in the blood-cell values. It gave a negative reaction in three subsequent palpebral tests also (see above), although there were shifts in the leucocyte number, the differential blood image, and the antibody titer after the tests. We consider this as a confirmation of a general theory (JACCARD 1956), according to which the tuberculin sensitivity of the skin does not run parallel with the antibody level.

Discussion of the results

The multifarious findings with respect to the relations between tuberculin concentration and skin reaction, with respect to the course of the HA titer and its dependence on the level of the tuberculin units, and with respect to the relations between the white blood-particle image and the tuberculin reaction + HA titer compel us to discuss the subject in separate sections. It should be remarked, however, that the results are of an orientation character owing to the small number of experimental animals involved, and thus permit statements of a limited scope only.

The fundamentals, significance, and nature of the tuberculin test were presented in a comprehensive manner for tuberculosis diagnostics by SPIESS (1959) and FREERKSEN (1960). According to FREERKSEN (1960), the body cells that acquired tuberculolipids are sensitive to particular protein forms. As a consequence of the depletion of tuberculoproteins, present in the low molecular-size form in tuberculin, a reaction develops at the location of the meeting. This reaction has the image of a tubercular granuloma that decomposes rapidly. The tuberculoprotein has a greater toxicity for the sensitized body than for the healthy one, and elicits at the location of its binding an accelerated and exaggerated inflammation reaction (HINDE and GELL 1952).

The tuberculin reaction was tried as a cutane test as early as 1914 on the lid of cattle (KLIMMER 1923). SCHROEDER initially recommended the lower lid (1938a); subsequently (1938b), the test was performed at the upper lid of monkeys for the purposes of tuberculosis diagnostics. The suprapalpebral tuberculin reaction appears to be favorable in Rhesus monkeys as a consequence of the absence of pigmentation and hairs on the lid (HARTMANN 1931); the ability of the skin to be shifted with respect to the substrate is also favorable. Thus, the results can be read without the need for subsequent capturing. Since these tuberculin injections are reported sometimes as subcutaneous ones (GRAHAM-JONES 1964; MAROIS et al 1961;

KENNARD and WILLNER 1941; KENNARD et al 1939) and some other times as intradermal or intracutaneous ones (ALLEN and KINARD 1958; FREMMING et al 1957; HABEL 1947), we performed the injection with hematoxylin lege artis in one titer to characterize the tuberculin deposit more closely. The dye was located on the basis of histological tests at the fibers of the loose connective tissue between the tarsal plate and the M. orbicularis oculi (Pars palpebralis), and the amount of liquid, 0.1 ml, formed slit spaces in this loose connective tissue (Fig. 9).

Fig. 9. Cross sections of the upper eyelid of a Rhesus
monkey. The left picture illustrates slit spaces (a) in
the loose connective tissue between Tarsus and M. orbi-
cularis oculi (Pars palpebralis) following intrapalpebral
injection of hematoxyline in lieu of tuberculin; the right
lid is untreated.

Since the injection takes place neither in the cutis nor in the subcutis, it is more accurate to say that the injection is an intrapelpebral one.

A scheme for the evaluation of the palpebral reaction was published by KENNARD and WILLNER (1941) and by BYWATER et al (1962). They distinguish four degrees of reaction, whereby a reaction with reddening without edema (stage 1) was initially still considered positive by KENNARD and WILLNER (1941), whereas BYWATER et al (1962) and other investigators consider only the simultaneous occurrence of reddening and swelling for a period of more than 24 hours as a true tuberculin reaction.

Dubious or questionable tests are as a rule not confirmed as tu-
bercular in the cross sections (KENNARD and WILLNER 1941). On the other
hard, there are reports of a number of falsely negative reactions also
(RUCH 1959). The most frequent reason for the failure of the tuberculin
test is, according to the first-named investigator, the negative finding
in the breakdown form of the tuberculosis.

The concentration and the nature of the tuberculin used, and
the site of the injection, are of decisive importance in case of monkeys
in tuberculosis diagnostics. Before the introduction of the palpebral
test, the intracutaneous, subcutaneous, and ophthalmal tests with KOCH-
type old tuberculin led to the conclusion that neither local nor general
reactions are of particular value (NOHLEN and SARVAN 1931) and that 'an
allergy is not provable by a tuberculin injection' (KALBFLEISCH and NOHLEN
1929). When the intrapalpebral test started (SCHROEDER 1938), on the other
hand, dilutions of the 'old tuberculin' (O. T.) to 1 mg and 10 mg per dose
were used with favorable results (KENNARD and WILLNER 1941). This tubercu-
lin concentration was subsequently increased from 5 mg O. T. (HABEL 1947)
or in the dilution of 1:20 O. T. (ALLEN and KINARD 1958) to 1:10 O. T.
(BENSON et al 1955; FREMMING et al 1957; DOLOWY et al 1958), where the
last named group of investigators employed undiluted old tuberculin. The
studies with P. P. D. also showed in the course of time an increase in con-
centration from 0.01 mg (FRANCIS 1956) to 0.2 mg (BYWATER et al 1962);
this seems to be justified by the studies conducted by the authors. The
intrapalpebral tests with 1 and 10 TE P. P. D in our own tuberculotic Rhe-
sus monkeys remained negative, whereas the same dose (10 TE) at the under-
arm caused the formation of measurable skin spots. Corresponding old tuber-
culin doses (1:1000 O. T.) led to negative reaction (DOLOWY et al 1958),
whereas they gave a positive reaction subsequently with 1:10 O. T. In tu-
berculin tests with 100 TE, there were again clear-cut skin swelling phe-
nomena at the arm, whereas palpebral reactions were lacking. This confirms
previous results for the lid test with 100 TE (TROLLDENIER 1964). Only at
an intrapalpebral tuberculin dose of 1000 TE is there a formation of clear-
ly identifiable tuberculin reaction. This finding approximately agrees with
the data reported by the above-named investigators, who in most instances
employed an equivalent old-tuberculin dilution of 1:10.

The skin reaction on 1000 TE was in many instances much more in-
tensive than the palpebral test, with a strong inflammation of the subsu-
taneous skin, and it lasted for six days. Even after 24 days the secondary
reactions were clearly detectable in some instances (Fig. 4). It should be
noted, however, that previous injections of 200 TE -- 24 days earlier --
caused a negative outcome of the cutaneous and lid test with 1000 TE in
two animals. In our judgment, this case represents a desensitization of
the skin receptors by earlier tuberculin injection, since tuberculin sen-
sitivity reoccurred later. A temporary desensitization is known for other
animals (MALLMANN et al 1964).

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Tuberculinization with 5000 TE in the eyelid showed, in contrast to the palpebral tests with 1000 TE, stronger reactions: there was repeated evidence of lid closure by edema formation. The reactions were visible without capturing the animals. In this case, too, there was noted an apparent desensitization in the form of a milder reaction with slight reddening and swelling in one animal that had reacted in a strongly positive manner in an intracutaneous injection with 5000 TE.

The possibility of a desensitization represents an important factor in repeated tests. If tuberculinizations are performed too frequently -- WHITE et al recommend monthly tests -- we believe that the skin reaction could be considerably weakened, as shown by our tests. JOHNSON (1961) found that a decrease in sensitivity in tuberculotic cattle at the injection site is compensated only after 30 days and lasts longer in non-infected animals, whereas MOENCH (1963) found no effect on size and development time of the skin reactions in guinea pigs by the frequency of testing.

Same as in the desensitizations observed, it is probably advantageous to extend the intervals between tuberculin tests in monkeys to more than one month. Without mentioning the possibility of a decrease in tuberculin sensitivity, KENNARD and WILLNER (1941) recommend three-monthly testing, as does HABEL (1947). An additional loss of sensitivity of the skin to tuberculin was observed in the breakdown phase of tuberculosis in monkeys by SCHROEDER (1938) and by us also in one Rhesus monkey (No. 1) in the stage of recovery. A decrease in tuberculin allergy is evident also in the course of a tuberculostatic treatment (FREMMING et al 1957). Although, according to WAGNER (1964) and other investigators, there is no spontaneous recovery from tuberculosis in monkeys, the five negative tuberculinizations following positive tests, together with the observed slight changes in the cross section of monkey No. 1, may indicate spontaneous recovery. The effect of a short INH treatment, that in other animals caused at most a stagnation in the development of tuberculosis, is not clearly evident in the individual case. The increase of the HA titer following tuberculinization in this animal appears to be also noteworthy in spite of the lack of a skin reaction.

Our electrophoretic investigations on three animals indicated no significant deviations in the individual fractions from the initial value after the tuberculin test with 5000 TE with daily control. Changes in the titer value during the first six days following the tuberculinization were not noticeable in the pherograms of the individual animals.

The effect on the antibody titer is prominent only after a tuberculinization with higher number of tuberculin units. Whereas in the case of 1 TE and TE, no titer changes were evident, a titer increase by one degree of dilution was evident in two animals after tuberculinization with 100 TE. After a tuberculin dose of 1000 TE, the titer increased within 3-6 days after tuberculinization by several titer stages.

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The strongest reaction was evident after using 5000 TE. After an initial titer loss of one or more degrees of dilution, there was later a titer increase to higher than the initial value. The decrease of titer can be   explained by the fact that at this concentration (5000 TE!) the tuberculin neutralizes a considerable portion of the humoral antibodies as a haptene. The subsequent increase in titer appears to be an expression of an activation of the 'tuberculotic occurrence.' These findings confirm the data of SCHLIESSER (1964), who was able to observe in calves that frequent tuberculinization acts as an excitation factor, capable of leading to the increased occurrence of humoral antibodies. MALLMANN et al (1964) observed a considerable increase in titer after 3-15 days in their studies on hogs. According to our experiences, one must consider an effect on the antibody level in the serum for approximately three weeks after tuberculinization with higher numbers of tuberculin units (TE). Only after more than this time has passed may the values obtained with the HAR be considered as uninfluenced. Furthermore, we found in monkey No. 6 a confirmation of the statement of WEIDMANN (1952) according to which no antibodies are evident in the terminal phase of tuberculosis infection. The titer observed the 7th day following depletion of 5000 TE must be considered as a reaction to the tuberculinization; a titer decrease was evident as early as the 14th day.

No reports are found in the literature so far with respect to the blood image of tuberculotic Rhesus monkeys. It appears to be unsuitable for supporting the diagnosis (TROLLDENIER 1964), since the established values are within the normal range that varies within wide limits (HALL 1929; FOX 1927). The physiological data of the hemogram of healthy Rhesus monkeys are subjected to a certain diurnal rhythm; during the late evening hours, there is an increase in the leucocyte number: this value is at its lowest during noontime. On the other hand, feeding does not appear to affect the values (digestion leucytosis)(FOX 1927). Differences in sex and age were not observed (SHUKERS et al 1938; POSPISIL and LAMBERTOVA 1965), although the erythrocyte number decreases during the menstrual cycle upon the onset of the ovulation (GUTHKELCH and ZUCKERMANN 1937). The percentage-wise distribution of the differential blood image differs very widely in the reports available; thus, for example, the percentage of the lymphocytes is given as 33 ± 12 (POSPISIL and LAMBERTOVA 1965), as 33-47 (FOX 1927), as 51.5 (HALL 1929), and as 59 ± 1.23 (SHUKERS et al 1938). Even in the original country of India, an average of 47.9% (MAJUNDER and DAS GUPTA 1944) and 61% (BILIMORIA 1931) of lymphocytes was reported. Accordingly, POSPISIL and LAMBERTOVA (1965) are justified in stating that the individual differences in the differential blood image are much wider than in other animals. This can be verified on the basis of the hemograms of the experimental animals.

The effects of the tuberculin or of the tuberculin reaction on the leucocyte number and the differential blood image occurred at 100 TE and higher doses. At this tuberculin concentration there was an observed increase in the leucocyte number by an average of 2000/cu mm during the

3rd day whereas at the next higher concentration of 100 TE per dose there
was a leucytosis on the 3rd day of approximately 4000/cu mm in all three
animals. The fact that this observation is probably of a random nature is
evident from the behavior of the white blood particle image after injec-
tion of 5000 TE. In this case, leucopenies of 2000/cu mm occurred in one
of the animals (No. 1) in two instances, and there also were two slight
leucocytoses of 800/cu mm after this injection dose. The other animals --
including monkey No. 5 -- showed a decrease in leucocyte number of between
600 and 3400/cu mm on the 3rd day p. inj.

More detailed shifts could be characterized by a daily blood tes-
ting after the tuberculin injection. Before a decrease in leucocyte num-
ber on the 3rd day, there was an increase in leucocyte number after 24
hours in two instances, and on the other hand, a leucopenia after 48 hours
of 7740/cu mm and a leucocytosis of 13,800/cu mm after 72 hours p. inj.

On the other hand, the composition of the differential blood image
showed in many instances a direct relation with the shifts in the leucocyte
number. A decrease in the leucocyte number often accompanied a relative in-
crease of the relative lymphocyte value and a corresponding loss of granu-
locyte number, while the HA titer increased at the same time (Fig. 1). In
an opposite development, the leucocyte number had the same course as the
lymphocyte number and the hemagglutination titer run parallel to the lympho-
cyte number. The change of the HA titer often led the corresponding lympho-
cyte shift. The increase of the number of lymphocytes with increasing anti-
body formation can be based, according to GRAU (1965) on the protein trans-
port function of the lymphocytes, the decomposition of which is the prere-
quisite for the formation of plasma cells.

Summary

Tuberculinizations were performed with increasing concentrations
on six Rhesus monkeys to establish the optimum tuberculin concentration in
the intrapelpebral and intracutaneous tuberculin reaction. At the same time,
the effect on the course of the hemagglutination titer, the blood cell va-
lues, and partly the serum protein fractions was observed, also.

Whereas there was evidence of a tuberculin sensitivity of the skin
of the underarm even at 10 and 100 TE (P. P. D.), the eyelid exhibited slight
reaction of tuberculin only with doses of 1000 TE and stronger reactions
with doses of 5000 TE.

It is recommended to perform the tuberculin test with 100 TE and
1000 TE on the underarm.

In the majority of cases, effects on the hemagglutination titer
were evident only in the tests with higher tuberculin doses. Whereas du-
ring the first few days there was a decrease in titer, this was followed
by an increase to higher than the initial values. The titer may be

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influenced by the tuberculin for as long as 3 weeks. The increase or
decrease of the titer is preceded by a similar change in the relative
lymphocyte number, whereas the change in the number in the segment-grained
granulocytes, running in the same direction, is accompanied by a parallel
shift of the number of total leucocytes.

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